

# 5 INACTIVATION, DNA DOUBLE STRAND BREAK INDUCTION AND THEIR REJOINING IN BACTERIAL CELLS IRRADIATED WITH HEAVY IONS

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Besides inactivation one of the major interest in our experiments is to study the primary damage in the DNA double strand breaks (DSB) after heavy ion irradiation [1]. These damages lead not only to cell death but also under repair activities to mutations. In further experiments we have investigated the inactivation with two different strains of *Deinococcus radiodurans* (R1, Rec 30) and the induction of DSB as well as the rejoining of DSB in stationary cells of *E. coli* (strain B/r) irradiated with radiations of different quality. In the latter case irradiations were done so that the cell survival was roughly at the same level. We measured the DSB using the pulse field gelelectrophoresis [2] which allows to separate between intact (circular) and damaged (linear) DNA. The irradiated cells were transferred to NB medium and incubated for different times to allow rejoining.

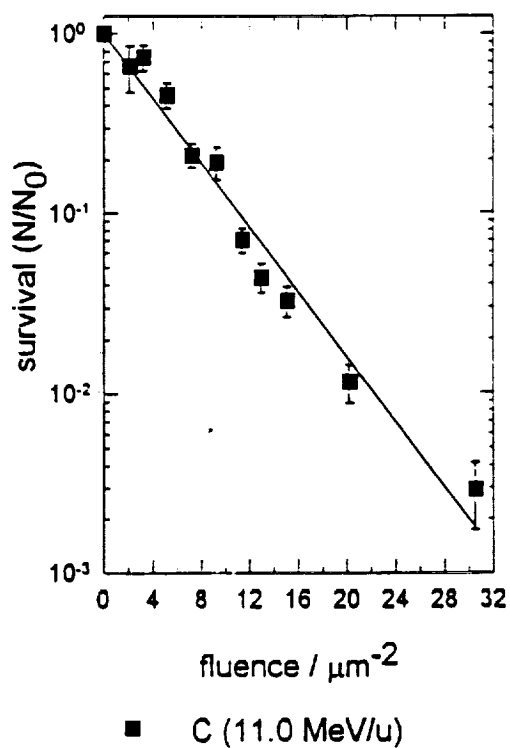
## INACTIVATION OF DEINOCOCCUS CELLS

The radiosensitive *Deinococcus*-mutant Rec 30 differs distinctly in the response to sparseley ionizing radiation in comparison to the wildtype *D. radiodurans* R1 [3]. The inactivation curve after X-irradiation is exponential, whereas the curve of the wildtype has a broad shoulder [4]. The radiosensitivity of Rec 30 expressed by the slope of the curve is about 20 times higher as that of the wildtype.

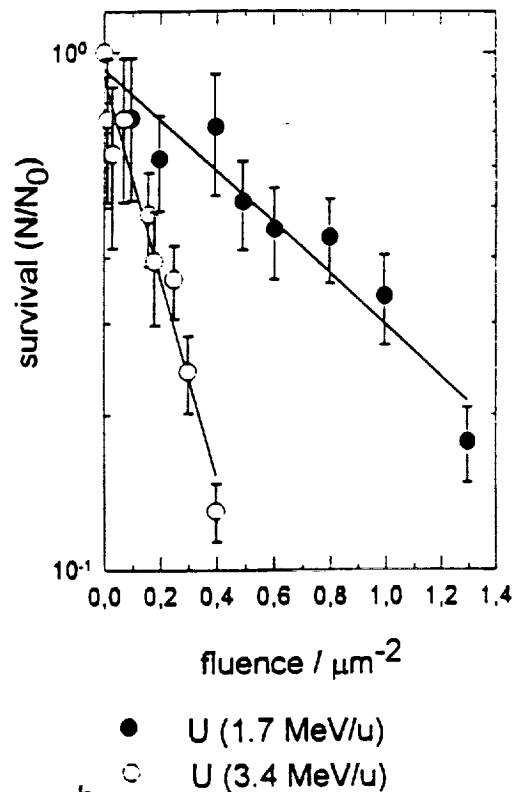
First experiments were made to study the survival of Rec 30 after heavy ion irradiation. Fig. 1 shows the inactivation curves of Rec 30 after C- and U-irradiation. The calculated cross sections from the survival curves (table 1) show significant differences in comparison to the wildtype, where the cross sections were calculated from the exponential part of the survival curve. In case of heavy ion irradiation we find that the cross sections of both strains differ by factors (C: 10, U: 2 and 6) that do not correspond with their X-ray radiosensitivities. After U-irradiation the differences of the obtained cross sections for the two strains become smaller with decreasing energy of the ion. The same effect was measured by Baltschukat [5] for different strains of *Bacillus subtilis*. In comparison to the inactivation cross sections of *E. coli* B/r and *Bacillus subtilis* measured by Schäfer et al. [6],[7] the data of Rec 30 fit well to the data of *E. coli* B/r while the data obtained for *D. radiodurans* R1 are similar to those of *Bacillus subtilis*.

Table 1: Inactivation cross sections ( $\sigma_i$ ) of *D. radiodurans* R1 and the mutant Rec 30 after irradiation with C (11,0 MeV/u) and U (1,7 and 3,4 MeV/u)

Ion	Energy MeV/u	<i>D. radiodurans</i> R1 $\sigma_i/\mu\text{m}^2$	Rec 30 $\sigma_i/\mu\text{m}^2$
C	11.0	$0.026 \pm 0.004$	$0.233 \pm 0.142$
U	1.7	$0.616 \pm 0.025$	$1.331 \pm 0.448$
U	3.4	$0.888 \pm 0.128$	$5.577 \pm 0.625$



a



b

Figure 1: Survival curves of the Deinococcus-mutant Rec 30 after irradiation with C-(a) and U-ions (b)

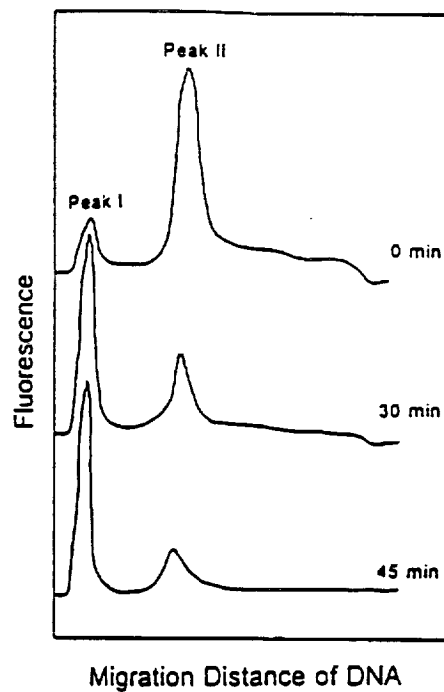


Figure 2: Scan profile of samples at selected repair times (Ni-ions:  $E = 650 \text{ MeV/u}$ , survival = 0.03)

## DSB INDUCTION AND REJOINING OF DSB IN E. COLI CELLS

In order to measure the rejoining kinetics of DSB we can determine the increase of intact DNA in the agarose plugs or the decrease of damaged DNA which is able to move in the gel. Fig. 2 gives the scan profiles at some repair times demonstrating both the increase of DNA content in peak I and the decrease in peak II, respectively.

The results described here (Fig. 3) are based on the analysis of the DNA content in the agarose plugs. This method is limited up to about 60 min because at larger times cell growing in the nutrient medium is not neglectable. For some repair experiments we found that the total amount of DNA decreases continuously for increasing times. One possible explanation could be that a number of cells undergo lysis when incubated in the NB medium. Therefore, we have determined the amount of DNA relative to the total amount for each sample and plotted as a function of the repair time in Fig. 3. The data qualitatively show that repair activity is started without any time delay for different ions varying in their energies and X-rays. Also, we find that the number of breaks rejoined per time interval depends on time and is remarkably reduced above 30 min. From the low number of experiments it is yet unclear whether the effects could be dependent also on the radiation quality. In principal, these results correspond to the break rejoining kinetics in other cell types.

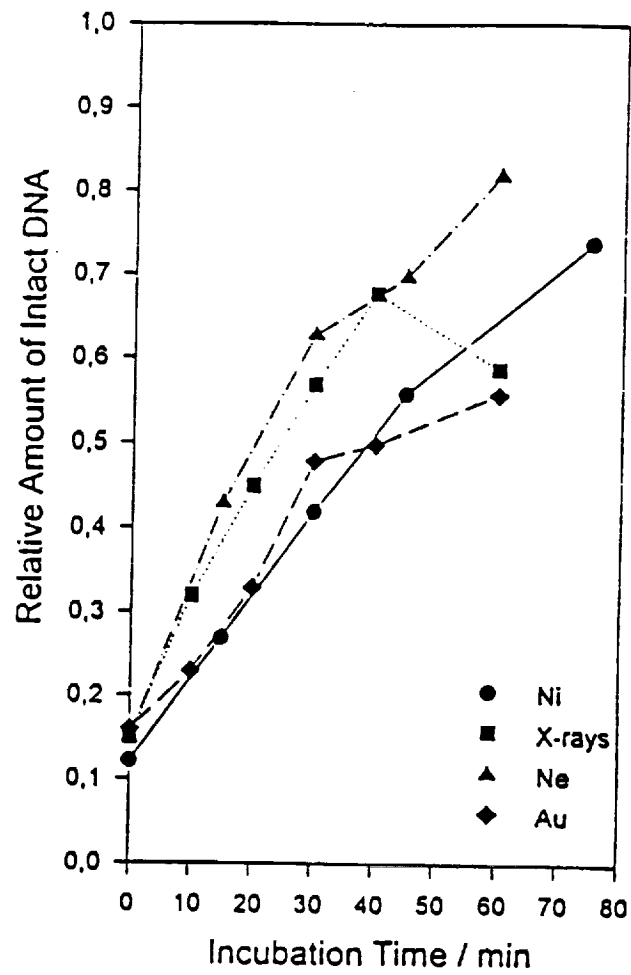


Figure 3: Dependence of the relative amount of DNA in the plug (Peak I) as a function of the repair time for different ions.

## References

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